Agricultural Water Management 202 (2018) 271–284

Contents lists available at ScienceDirect

Agricultural Water Management

journal homepage: www.elsevier.com/locate/agwat

Arbuscular mycorrhizal symbiosis and salicylic acid regulate aquaporins and root hydraulic properties in maize plants subjected to drought

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ARTICLE INFO

Article history:
Received 20 July 2017
Received in revised form
28 November 2017
Accepted 15 December 2017
Available online 24 December 2017

Keywords:
Aquaporin
Arbuscular mycorrhizal symbiosis
Drought
Root hydraulic conductivity

ABSTRACT

Climate change is leading to the intensification of drought effects worldwide, which considerably reduce crop production. A better understanding of the drought-tolerance mechanisms would lead into a more productive agriculture. The arbuscular mycorrhizal (AM) symbiosis has been shown to improve plant tolerance to drought. Salicylic acid (SA) is a phenolic compound involved in many aspects of plant growth and development. Apart from its role in biotic interactions, it is also involved in the regulation of important plant physiological processes, including plant water relations under stressful conditions. However, despite the importance of SA in plant physiology and in AM colonization, little is known about its effect on regulation of root water transport. Thus, the aim of this work was to study the combined effect of AM symbiosis and SA on root hydraulic properties under drought stress, with special focus on how these factors can alter radial root water transport pathways through aquaporin regulation. Also, the crosstalk between SA and other phytohormones was taken into account. Results showed that the AM symbiosis modifies root hydraulic responses to drought episodes. Under these conditions, AM plants showed increased Lpr and Lo. Exogenous SA application decreased Lpr and Lo under drought. SA modulation of water conductivity could be due to a fine-regulation of root aquaporins (as *ZmPIP2;4* or *ZmTIP1;1*). Furthermore, SA application differently modulated the percentage of water flowing by the apoplastic pathway, decreasing its contribution to total root water flow in AM plants and increasing it in non-AM plants. An intricate relationship between Lpr, aquaporins and endogenous levels of SA, ABA and jasmonic acid was observed. Future studies should explore more in detail the crosstalk mechanism between these hormones in the regulation of water transport in AM roots, in order to better understand the mechanism through which the AM symbiosis copes with drought stress.

1. Introduction

Climate change is leading to the intensification of drought effects and cultivable soils are progressively drying worldwide (Trenberth et al., 2014), with more often drought events that considerably reduce crop production (Lesk et al., 2016). Agricultural drought reduces plant growth and affects essential plant physiological and biochemical processes as stomatal conductance, transpiration, root water uptake, photosynthesis or membrane functions. It also increases the production of reactive oxygen species (ROS), producing oxidative stress that damages cells and even leads to plant death (Hasanuzzaman et al., 2014). Thus, a better understanding of the mechanisms that help plants to improve their water status during water stress would lead into a more productive agriculture. Phytohormones play essential roles and coordinate different signalling pathways during abiotic stress responses (Wani et al., 2016). Among these, salicylic acid (SA) is a phenolic compound involved in many aspects of growth and plant development as well as in the regulation of the response to different abiotic and biotic stresses (Khan et al., 2015; Miura and Tada, 2014). Salicylic acid has been studied mainly in relation to plant-pathogen interactions since it has the ability to induce systemic acquired resistance to different pathogens in plants (Gunes et al., 2007). Indeed, it coordinates the plant's defence against biotrophic pathogens (Lu, 2009)

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https://doi.org/10.1016/j.agwat.2017.12.012
0378-3774/

and Foo et al. (2013) suggested that SA might also have a role during arbuscular mycorrhizal (AM) colonization. Previous studies point in this direction, with a short-lived rise in SA levels during the early stages of AM colonization (Blilou et al., 1999). Herrera-Medina et al. (2003) showed that the rate of AM colonization was affected by the SA content. They found that transgenic plants with reduced SA levels exhibited a more rapid AM colonization while wild-type plants with constitutive SA biosynthesis retarded AM colonization of roots, although the final level of colonization was unaltered.

Apart from this role in biotic interactions, SA is also involved in the regulation of important plant physiological processes such as nitrogen metabolism, photosynthesis, antioxidant defense system and plant water relations under stress conditions and thereby provides protection in plants against abiotic stresses (Faried et al., 2017; Khan et al., 2015). SA has been found to improve plant tolerance to salt stress (Jini and Joseph, 2017; Miura and Tada, 2014) and to affect plant physiology in maize plants subjected to salinity (Gunes et al., 2007). Indeed, exogenous SA may induce stomatal closure (Miura and Tada, 2014), regulates biosynthesis of osmolytes (Li et al., 2016; Misra and Saxena, 2009) and increases antioxidative defenses in stressed tissues (Nazar et al., 2011). However, SA is thought to interact in a complex way with other hormonal compounds such as ethylene (Gharbi et al., 2016). Thus, its effects on plant physiology can be direct or indirect, through alteration of other plant hormones. Finally, SA influences plant functions in a dose dependent manner, where induced or inhibited plant functions can be possible with low and high SA concentrations, respectively (Khan et al., 2015).

There are increasing evidences of enhanced drought tolerance when exogenous SA is applied (Alam et al., 2013; Miura and Tada, 2014; Li et al., 2016). However, this regulation is orchestrated in a complex cross-talk between different phytohormones (auxins, cytokinins, ABA, gibberellins) under optimal and stressful conditions (Munné-Bosch and Müller, 2013). On the other hand, AM fungi (which establish a mutualistic relationship with most crop plants) have been described to improve water and nutrient uptake, enhancing tolerance to abiotic stresses such as drought (Ruiz-Lozano et al., 2012) being a possible alternative to the use of inorganic fertilizers (Zoppellari et al., 2014). This amelioration is achieved by allowing plants the access to distant water from the soil, and by altering root hydraulic properties (Bárzana et al., 2012). Water transport in roots, according to the composite model (Steudle and Peterson, 1998) occurs as the sum of three pathways: apoplastic (via the cell wall continuum), symplastic (via plasmodesmata) and transcellular (across the cell membranes). The last two pathways cannot be differentiated empirically, being reduced to the so-called cell-to-cell pathway. Aquaporins play an important regulatory role in this last pathway, and within this protein family, water channel activity is mainly found in the PIP2 subfamily (Maurel et al., 2008). By measuring root hydraulic conductivity (Lpr), root water transport capacity can be estimated, providing information on plant water status and water mobilization capacity of roots.

It is known that under non-stressful conditions the radial water flow is mainly apoplastic, following the hydrostatic gradient created by transpiration. However, when transpiration is restricted (as under drought), water goes mainly by the cell-to-cell pathway following an osmotic gradient between soil solution and xylem sap. Thus, relative contribution of these two pathway to overall water uptake or hydraulic conductivity may change substantially (Hachez et al., 2006; Martínez-Ballesta et al., 2003; Vadez et al., 2013) and, under drought conditions, root hydraulics is adjusted by switching between both pathways (Ranathunge et al., 2004). It is expected that aquaporins play a key role in the regulation of water flow in plants under conditions of water limitation, affecting important parameters such as the root hydraulic conductivity (Hachez et al., 2006; Zarrouk et al., 2016). Moreover, there is growing evidence that the contribution of aquaporin-mediated water transport to root water uptake is much larger than previously thought, even under conditions of high transpiration (Knipfer and Fricke, 2010, 2011).

Previous studies have investigated the effects of the AM symbiosis on water pathways in the roots of host plants, combined with the use of an inhibitor of aquaporins activity (Bárzana et al., 2012). Results showed that roots of AM plants enhanced significantly the water circulating by apoplastic pathway as compared to non-AM plants, both under well-watered and under drought stress conditions. Data also showed that the presence of the AM fungus in the roots of the host plants could modulate the switching between cell-to-cell and apoplastic water transport pathways. This was interpreted as a way to provide higher flexibility in the response of AM plants to water shortage according to the demands from the shoot (Bárzana et al., 2012). Other recent evidences suggest that the modulation of ABA, auxins and/or SA levels in the host plant may contribute to this switching between water pathways mediated by the AM fungus (Calvo-Polanco et al., 2014; Sánchez-Romera et al., 2016). Indeed, ABA was found to increased Lpr at root cortical cell and organ levels in maize, facilitating water uptake under water limiting conditions (Hose et al., 2000) and ABA was identified as a possible aquaporin regulator (Boursiac et al., 2008; Wan et al., 2004). Studies in Arabidopsis indicated that indole acetic acid (IAA) acts through an Auxin Response Factor 7 (ARF7)-dependent path to inhibit the expression of most PIPs at both transcriptional and translational levels (Péret et al., 2012). Similarly, SA down regulates PIP aquaporins and root hydraulic conductivity by a ROS-mediated mechanism which provoked membrane internalization of PIP aquaporins (Boursiac et al., 2008).

Despite the importance of SA in plant physiology and in AM colonization, as well as its putative role under drought conditions, little is known about its effect on root hydraulic conductivity and regulation of water transport in roots, and to the best of our knowledge, studies about the combined effect of exogenous SA application and AM symbiosis are lacking. Thus, the aim of this research was to study the combined effect of AM symbiosis and SA on root hydraulic properties under drought stress, being specially focused on how these factors can alter radial root water transport pathways through aquaporin regulation. For that, we applied exogenous SA or an inhibitor of its biosynthesis (2-aminoindan-2-phosphonic acid, AIP; Pan et al., 2006). Also, the crosstalk between SA and other plant hormones under the former conditions will be discussed. The results of this study could lead to a better understanding of water uptake mechanisms and plant tolerance to drought when the AM fungus is present, increasing our knowledge of its effect on plant water balance.

2. Material and methods

2.1. Experimental design

The experiment consisted of a factorial design with three factors: (1) inoculation treatment, with non-inoculated control plants (C) and plants inoculated with the AM fungus *Rhizophagus irregularis*, strain EEZ 58 (Ri); (2) chemical treatment, so that one group of each inoculation treatment was maintained without hormone (untreated), another group of plants was treated with salicylic acid (SA), and the last group was treated with 2-aminoindan-2-phosphonic acid (AIP), as inhibitor of SA biosynthesis; (3) watering treatment so that half of the plants were grown under well-watered (WW) conditions throughout the entire experiment and the other half was subjected to drought stress for 15 days before harvest (DS). The different combination of these factors gave a total of 12

treatments. Each treatment had 10 replicates, giving a total of 120 plants.

2.2. Biological material and growth conditions

A loamy soil was collected at the grounds of IFAPA (Granada, Spain), sieved (2 mm), diluted with quartz-sand (<1 mm) (1:9, soil:sand, v/v) and sterilized by steaming (100 °C for 1 h on 3 consecutive days). The soil had a pH of 8.1 (water); 0.85% organic matter, nutrient concentrations (mg kg^{-1}): N, 1; P, 10 ($NaHCO_3$-extractable P); K, 110. The soil texture comprised 38.3% sand, 47.1% silt and 14.6% clay.

Seeds *of Zea mays* L. cultivar PR34B39 were provided by Pioneer Hi-Bred, Spain (DuPont Pioneer Corporation). Maize plants were grown in 1L pots filled with 1250 g of a mixture of soil/sand (1:9) for 8 weeks. At the time of planting, half of the plants were inoculated with ten grams of mycorrhizal inoculum from *Rhizophagus irregularis* (Schenck and Smith), strain EEZ 58. Mycorrhizal inoculum consisted of soil, spores, mycelia and infected root fragments. Non inoculated plants received a 5 mL aliquot of a filtrate (<20 μm) of the AM inoculum in order to provide the natural microbial population free of AM propagules.

Plants were grown for 8 weeks in a greenhouse at 19/25 °C, 16/8 light/dark period, 50–60% relative humidity and an average photosynthetic photon flux density of 800 μmol m^{-2} s^{-1}, as measured with a light meter (LICOR, Lincoln, NE, USA, model LI-188B). Plants were irrigated three times per week with 50 mL of Hoagland nutrient solution (Hoagland and Arnon, 1950) modified to contain 25% P in order to avoid AM symbiosis inhibition. The same amount of water was applied on alternate days. A drought stress treatment was applied for the last 2 weeks, by irrigating plants with half the water/Hoagland volume of well-watered ones (25 mL vs. 50 mL). This water stress was similar as in a previous work with similar experimental design (Quiroga et al., 2017). It could be considered as a severe stress as evidenced by a drop of stomatal conductance by around 75% (Table 1).

Salicylic acid 20 μM and AIP 75 μM were applied with the nutrient solution 6 h before harvesting. Dose of the phytohormone and its inhibitor, as well as, the exposure time needed to affect root hydraulic conductivity were established in previous experiments ranging from 20 to 150 μM SA, 25–100 μM AIP, and exposure times of 1 h, 6 h, 12 h and 24 h.

2.3. Measurements

2.3.1. Biomass production and symbiotic development

At harvest the shoot and root system of ten replicates per treatment were collected and used for fresh weight recording. Then, 5 replicates per treatment were dried in a hot-air oven at 70 °C for 2 days and dry weights were recorded. The other 5 replicates were immersed in liquid nitrogen and stored at −80 °C until they were used.

Roots of maize were stained according to Phillips and Hayman (1970), in order to differentiate fungal structures. The extent of mycorrhizal colonization was calculated according to the gridline intersect method (Giovannetti and Mosse, 1980) in five replicates per treatment.

2.3.2. Stomatal conductance

Stomatal conductance (*gs*) was measured two hours after the onset of photoperiod in the second youngest leaf from 10 plants per treatment with a porometer system (Porometer AP4, Delta-T Devices Ltd, Cambridge, UK) following the manufacter's instructions. Measurements were taken one day before harvest.

2.3.3. Photosynthetic efficiency

The efficiency of photosystem II was measured with FluorPen FP100 (Photon Systems Instruments, Brno, Czech Republic), which allows a non-invasive assessment of plant photosynthetic performance by measuring chlorophyll a fluorescence. FluorPen quantifies the quantum yield of photosystem II as the ratio between the actual fluorescence yield in the light-adapted state (FV') and the maximum fluorescence yield in the light-adapted state (FM'), according to Oxborough and Baker, 1997. Measurements were taken in the second youngest leaf of 10 different plants of each treatment one day before harvest.

2.3.4. Membrane electrolyte leakage

Leaf electrolyte leakage was determined in 10 plants per treatment. Leaf samples were washed with deionized water to remove surface-adhered electrolytes. The samples were placed in 15 mL falcon tubes containing 10 mL of deionized water and incubated at 25 °C on a rotary shaker (at 100 rpm) during 3 h, and the electrical conductivity of the solution (E_0) was determined using a conductivity meter (Mettler Toledo AG 8603, Switzerland). Samples were then placed at −80 °C for 2 h. Subsequently, tubes were incubated again at room temperature under smoothly agitation and the final electrical conductivity (E_f) was obtained after 3 h under these conditions. The electrolyte leakage was defined as follows: $[(E_0 - E_{water})/(E_f - E_{water})]$ X 100, where E_{water} is the electrical conductivity of the deionized water used to incubate the samples.

2.3.5. Osmotic root hydraulic conductivity (Lo)

Lo was measured at noon on detached roots exuding under atmospheric pressure by the free exudation method (Benabdellah et al., 2009). Under these conditions, water is only moving following an osmotic gradient. Therefore, the water would be moving through the cell-to-cell path (Steudle and Peterson, 1998). The exuded sap was collected after 2 h and weighed. The osmolarity of the exuded sap and the nutrient solution was determined using a cryoscopic osmometer and used for *Lo* calculation, according to Aroca et al. (2007). Lo was calculated as $Lo = Jv/\Delta Y$, where Jv is the exuded sap flow rate and $\Delta\Psi$ the osmotic potential difference between the exuded sap and the nutrient solution where the pots were immersed. Measurements were carried out 6 h after starting the chemical treatment.

2.3.6. Hydrostatic root hydraulic conductivity (Lpr)

The *Lpr* was determined at noon in five plants (n = 5) per treatment with a Scholander pressure chamber, 6 h after starting the chemical treatment and following the method described by Bárzana et al. (2012). A gradual increase of pressure (0.2, 0.3 and 0.4 MPa) was applied at 2-min intervals to the detached roots. Sap was collected after 2 min at the three pressure points. Sap flow was plotted against pressure, with the slope being the root hydraulic conductance (*L*) value. Lpr was determined by dividing *L* by root dry weight (RDW) and expressed as mg H_2O g RDW^{-1} MPa^{-1} h^{-1}. Aliquots of the collected sap were used for subsequent hormonal determination.

2.3.7. Relative apoplastic water flow

Relative changes in apoplastic water flux were estimated using light green dye (light green SF yellowish; Sigma-Aldrich Chemical, Gillingham, Dorset; colour index 42095, molecular weight 792.85 g mol^{-1}), which has the ability to move apoplastically but not symplastically (López-Pérez et al., 2007). Detopped root systems were immersed in 250 μmol L^{-1} light green solution inside the pressure chamber 5 min before pressure application and kept in this solution during measurement. Sap was collected after 2 min at 0.2, 0.3 and 0.4 MPa in a Scholander pressure chamber. At the end,

Table 1
Percentage of mycorrhizal root length, shoot dry weight (SDW), root dry weight (RDW), electrolyte leakage (EL), stomatal conductance (gs) and photosystem II efficiency in the light-adapted state (ΔFv/Fm') in maize plants inoculated or not with the AM fungus *Rhizophagus irregularis* and submitted to two water regimes (well-watered-WW- or drought stress DS). Data represents the means of six values ± SE for mycorrhization, twelve values ± SE for gs, ΔFv/Fm' and EL; and thirty values ± SE for SDW and RDW. Different letter indicates significant differences between treatments ($p < 0.05$) based on Duncan's test.

	Mycorrhization (%)	SDW (g plant^{-1})	RDW (g plant^{-1})	EL (%)	gs (mmol H_2O m^{-2} s^{-1})	ΔFv/Fm'
WW non-AM	n.d.	6.85 ± 0.14 b	7.42 ± 0.46 b	10.07 ± 1.07 b	88.90 ± 15.77 a	0.700 ± 0.004 a
WW AM	64.8 ± 3.2 a	7.77 ± 0.20 a	10.78 ± 0.85 a	8.24 ± 0.87 b	88.88 ± 7.35 a	0.683 ± 0.011 a
DS non-AM	n.d.	4.23 ± 0.09 d	4.91 ± 0.16 c	18.06 ± 3.04 a	27. 09 ± 2.42 b	0.702 ± 0.005 a
DS AM	65.9 ± 5.7 a	4.71 ± 0.08 c	5.99 ± 0.26 c	8.09 ± 1.51 b	31.31 ± 3.47 b	0.704 ± 0.003 a

the concentration of the dye in the whole collected sap was determined immediately at 630 nm (Bárzana et al., 2012). The average baseline fluorescence value in the nutrient solution before addition of the dye was subtracted to the values obtained after adding the dye and in the collected sap. The percentage of apoplastic pathway was calculated from the ratio between dye concentration in the sap flow and in the nutrient solution. The concentration of dye in the nutrient solution of each treatment was considered to be 100%.

2.3.8. Sap and root hormonal content

In sap, IAA, ABA, SA and JA were analysed according to Albacete et al. (2008) with some modifications. Briefly, xylem sap samples were filtered through 13 mm diameter Millex filters with 0.22 μm pore size nylon membrane (Millipore, Bedford, MA, USA). Ten μl of filtrated extract were injected in a U-HPLC–MS system consisting of an Accela Series U-HPLC (ThermoFisher Scientific, Waltham, MA, USA) coupled to an Exactive mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) using a heated electrospray ionization (HESI) interface. Mass spectra were obtained using Xcalibur software version 2.2 (ThermoFisher Scientific, Waltham, MA, USA). For quantification of the plant hormones, calibration curves were constructed for each analysed component (1, 10, 50, and 100 μg L^{-1}).

In plant roots, IAA, ABA, SA, JA and JA-Ile were analysed using high-performance liquid chromatography-electrospray ionization-high-resolution accurate mass spectrometry (HPLC-ESI_HRMS) as described in Ibort et al. (2017).

2.3.9. PIP aquaporins abundance and phosphorylation status

Microsomal fraction isolation and ELISA were performed as described previously by Calvo-Polanco et al. (2014). We used five different primary antibodies (at a dilution of 1:1000), two antibodies that recognize several PIP1 s and PIP2s, and three antibodies that recognize the phosphorylation of PIP2 proteins in the C-terminal region: PIP2A (Ser-280), PIP2 B (Ser-283) and PIP2C (Ser-280/Ser-283) (Calvo-Polanco et al., 2014).

2.3.10. Gene expression analysis through quantitative real-time RT-PCR

Total RNA was extracted from five biological replicates of maize roots harvested 8 weeks after sowing and conserved at −80 °C prior to use. Isolation was carried out by a phenol/chloroform extraction method followed by precipitation with LiCl (Kay et al., 1987). The integrity of RNA was checked electrophoretically and quality assessment of total RNA was measured with NanoDrop (Thermo Scientific™;NanoDrop 1000). First-strand cDNA was synthesized using 1 μg of purified RNA with the Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase (Thermo Scientific™), according to the manufacter's protocol. To rule out the possibility of a genomic DNA contamination, all the cDNA sets were checked by running control PCR reactions with aliquots of the same RNA that have been subjected to the DNase treatment but not to the reverse transcription step.

The expression of a group of maize aquaporins previously selected as regulated by the AM symbiosis (Bárzana et al., 2014; Quiroga et al., 2017) was studied by real-time PCR by using iCycler system (Bio-Rad, Hercules, CA, USA) adjusting protocols to optimize the PCR reaction to each gene. The primer sets used to amplify each aquaporin gene were designed in the 3′ and 5′ untranslated regions of each gene in order to avoid unspecific amplification of the different aquaporin genes (Bárzana et al., 2014; Hachez et al., 2006). Polymerase chain reactions were performed in a 96-well plate with an iCycler 5 system (Bio-Rad, Hercules, California, USA), using KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal (KAPABIOSYSTEMS, Boston, Massachusetts, United States). The following standard thermal profile was used for all PCR reactions: Enzyme activation (95 °C for 3 min), denaturation, annealing and extension cycles repeated 40 times (95 °C for 25 s, 60 °C for 25 s, 72 °C for 30 s) and dissociation curve (70 °C for 2 min, 55° C for 10 s).

The Elongation Factor 1 (*gi:2282583*) was used as reference gene for normalization, as it was the best-performing reference gene under the specific growing conditions. The relative abundance of transcripts was calculated from three biological and two technical replicates by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Negative controls without cDNA were used in all PCR reactions.

2.4. Statistical analysis

Within each watering regime, data were analysed using SPSSStatistics (version 23, IBM Analytics) and subjected to analysis of variance (ANOVA) with inoculation treatment and chemical treatment as sources of variation. Post-hoc comparisons with Duncan test were used to find out differences between means at α=0.05. Correlations between the different parameters were performed by calculating the Pearson correlation coefficients.

3. Results

3.1. Root mycorrhization, plant growth and ecophysiological paramaters

Plants inoculated with *Rhizophagus irregularis* (AM) presented around 65% of mycorrhizal root length, showing no significant differences between water treatments, whereas non-inoculated plants did not show AM colonization (Table 1).

Shoot and root dry weight decreased significantly by 40% in average due to drought stress treatment, but AM plants maintained higher plant dry weight than non-AM ones, regardless of water regime (Table 1). Thus, under well-watered conditions the shoot dry weight was 13% higher in AM plants and under drought stress conditions the increase was by 11%. Membrane electrolyte leakage (EL) was significantly enhanced by 79% in non-AM plants after drought stress. In contrast, AM plants maintained steady state levels as compared to well-watered treatments (Table 1). Stomatal conductance (*gs*) was significantly reduced after two weeks of water limited conditions both in AM and in non-AM plants (Table 1). No differences were found in the efficiency of photosystem II due to water availability or AM fungal inoculation (Table 1).

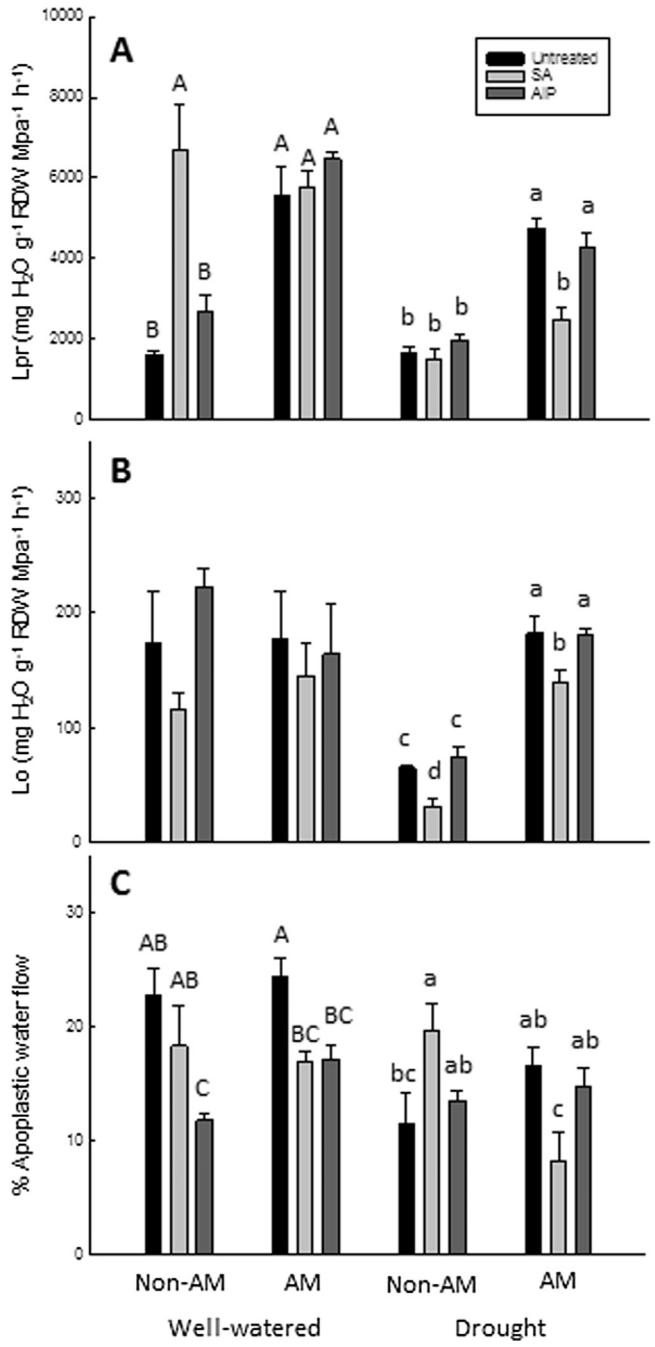

Fig. 1. Hydrostatic root hydraulic conductivity (Lpr) (A), osmotic root hydraulic conductivity (Lo) (B) and relative apoplastic water flow (C) in maize plants inoculated (AM) or not (Non-AM) with the AM fungus *Rhizophagus irregularis* and submitted to two water regimes (well-watered or drought stress). Plants remained untreated or received exogenous salicylic acid (SA) or an inhibitor of SA biosynthesis (AIP). Data represents the means of five values ± SE. Different letter indicates significant differences between treatments ($p < 0.05$) based on Duncan's test for well-watered (uppercase) and drought stressed (lowercase) plants. The absence of letters indicates that no significant differences among treatments were found.

3.2. Hydrostatic and osmotic root hydraulic conductivities and percentage of apoplastic water flow

Under well-watered conditions Lpr was enhanced significantly by SA application in non-AM plants (Fig. 1A). AM inoculation also enhanced Lpr as compared to non-AM plants, but no further enhancement was observed in these plants due to SA application. The application of AIP in non-AM plants maintained Lpr values similar to control untreated plants.

Under drought stress conditions Lpr values were also higher in AM plants than in non-AM plants (Fig. 1A). The application of SA inhibited Lpr by 47% in AM plants, while the application of AIP maintained steady-state Lpr values. In non-AM plants Lpr exhibited the lowest values and no effects of either SA or AIP were observed.

Under well-watered conditions Lo resulted unaffected by AM inoculation, SA or AIP application (Fig. 1B). Under drought stress conditions Lo values where always considerably and consistently higher in AM plants than in non-AM plants. The application of SA inhibited Lo both in AM plants (by 23%) and in non-AM plants (by 51%). The application of AIP maintained steady-state Lo values in both kinds of plants.

The percentage of apoplastic water flow under well-watered conditions was similar in AM and non-AM plants (Fig. 1C). The SA application reduced significantly this value only in AM plants, while the application of AIP reduced this value both in AM and non-AM plants. Under drought stress conditions the application of SA had contrasting effects in AM and non-AM plants. Thus, SA enhanced by 71% the percentage of apoplastic water flow in non-AM plants, but decreased it by 50% in AM plants. Again, the application of AIP did not affect the apoplastic water flow as compared to untreated plants.

3.3. Expression of maize aquaporin genes

In this study we analyzed the expression of 8 maize aquaporin genes shown in a previous report to be consistently regulated by the AM symbiosis under drought stress (Quiroga et al., 2017). In well-watered non-AM plants *ZmPIP1;1* aquaporin relative expression level was unaltered by SA application but it showed a significant increase due to AIP treatment (Fig. 2A). In such conditions, AM inoculation resulted in *ZmPIP1;1* expression drop in the case of untreated plants and plants treated with AIP. On the other hand, droughted non-AM plants featured a significant decrease in *ZmPIP1;1* expression in presence of external SA but no effect of AM inoculation was detected.

Similar results were obtained for *ZmPIP2;2* relative expression of well-watered plants (Fig. 2B), no effect after SA application but significant decreases due to AM inoculation in untreated plants and in plants treated with AIP. Under drought conditions SA and AIP led to a significant decline in *ZmPIP2;2* relative expression in non-AM plants, whereas no effect of AM inoculation was featured.

Under well-watered conditions *ZmPIP1;3* relative expression was unaltered, but when plants grew under water limited conditions, its expression was reduced in non-AM plants after AIP application (Fig. 2C). AM inoculation also significantly decreased its expression but only in untreated plants (Fig. 2C).

When analyzing the *ZmPIP2;4* aquaporin mRNA it was highlighted that when non-AM plants grew well irrigated SA application decreased its expression, but AIP maintained steady-state expression level as comparted to control untreated plants (Fig. 2D). In such conditions, AM reduced *ZmPIP2;4* relative expression in the case of untreated well-watered plants or after AIP treatment. Under drought stress conditions no chemical effect was shown and AM inoculation only provoked *ZmPIP2;4* expression to drop in the case of SA treated plants. Interestingly, a similar pattern was found when comparing *ZmTIP1;1* relative expression (Fig. 2E). Fully-watered non-AM plants also showed a significant decrease in gene expression after SA application and a significant increase when treated with AIP. AM symbiosis contributed to *ZmTIP1;1* relative expression drop in untreated or AIP-treated plants. Contrariwise, none of the studied factors altered *ZmTIP1;1* expression under drought stress conditions.

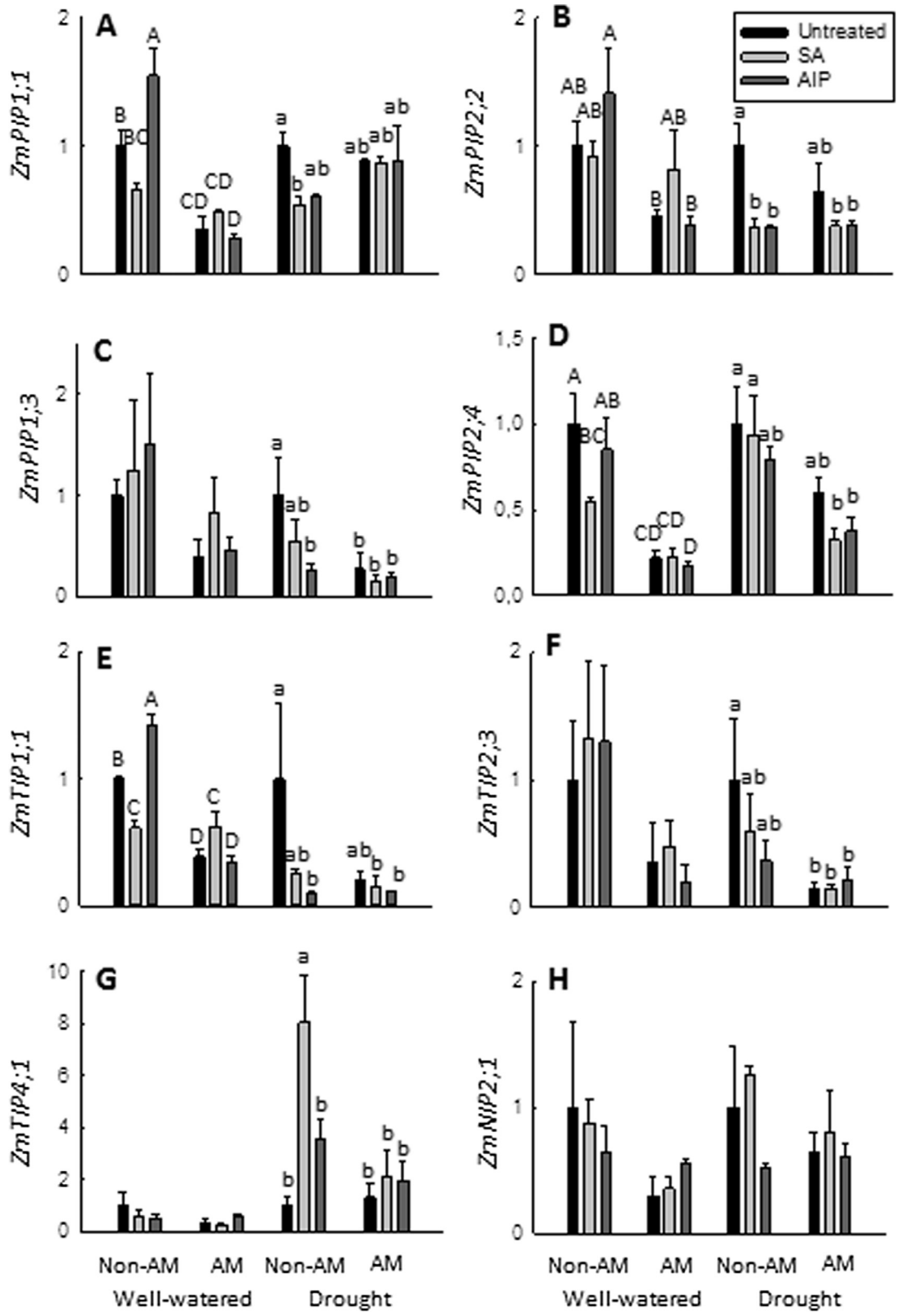

Fig. 2. Relative expression of *ZmPIP1;1* (A), *ZmPIP2;2* (B), *ZmPIP1;3* (C), *ZmPIP2;4* (D), *ZmTIP1;1* (E), *ZmTIP2;3* (F), *ZmTIP4;1* (G), and *ZmNIP2;1* (H) genes in maize plants inoculated (AM) or not (Non-AM) with the AM fungus *Rhizophagus irregularis* and submitted to two water regimes (well-watered or drought stress). Plants remained untreated or received exogenous salicylic acid (SA) or an inhibitor of SA biosynthesis (AIP). Data represents the means of five values ± SE. Different letter indicates significant differences among treatments ($p < 0.05$) based on Duncan's test for well-watered (uppercase) and drought stressed (lowercase) plants. The absence of letters indicates that no significant differences among treatments were found.

Well-watered plants did not show significant differences regarding *ZmTIP2;3* relative gene expression, but under water limited conditions a significant inhibition was observed due to AM symbiosis in untreated plants (Fig. 2F).

Well-watered plants did not feature changes regarding *ZmTIP4;1* relative gene expression (Fig. 2G). However, under drought stress, SA application led to gene expression enhancement in non-AM plants, which were maintained under steady-state levels after AIP treatment. This behaviour was not observed when plants were inoculated with *R. irregularis,* since no changes were detected in *ZmTIP4;1* relative gene expression after chemical treatment (Fig. 2G).

Concerning the relative expression of *ZmNIP2;1* aquaporin no significant alterations were featured due to the studies treatments, regardless of the water regime (Fig. 2H).

3.4. Aquaporin protein abundance

PIP1 and PIP2 aquaporin proteins abundance were measured. Besides, it was quantified the PIP2 phosphorylation state in roots as aquaporin water channel activity depends on this post-transcriptional modification. In this line, the content of PIP2 protein phosphorylated at Ser-280 (PIP2A), at Ser-283 (PIP2B) and double phosphorylated at Ser-280 and Ser-283 (PIP2C) was also quantified. In addition, specific antibodies were used to quantify the abundance of ZmTIP1;1 and ZmPIP2;4 proteins.

Under well-watered conditions PIP1 protein abundance was unaffected by chemical treatment but significantly decreased due to AM fungus inoculation (Fig. 3A). However, when plants grew under drought stress conditions no significant alteration of PIP1 proteins in roots was registered as result of the studied factors alone or in combination.

Regarding the PIP2 proteins abundance, it is remarkable that fully-irrigated plants showed no differences due to chemical treatment or fungal inoculation (Fig. 3B). Nevertheless, non-AM droughted plants increased PIP2 proteins content after SA application.

Phosphorylated PIP2 proteins at Ser-280 (PIP2A) featured no relevant changes due to chemical treatment under well-watered conditions (Fig. 3C), but a significant decrease after AM inoculation in untreated plants was observed. In the case of drought stressed treatments, no changes were observed as result of chemical treatment or AM symbiosis.

PIP2 proteins phosphorylated at Ser-283 (PIP2B) of fully-watered plants showed no significant differences because of the chemical treatment (Fig. 3D), but a significant drop due to AM inoculation in the case of SA-treated plants was observed. PIP2 B protein of plants subjected to drought stress did not feature any significant change related to chemical treatments or AM symbiosis.

Interestingly, PIP2C (PIP2 proteins phosphorylated at Ser-280 and Ser-283) showed a similar pattern than PIP2B (Fig. 3E). In both cases, when plants grew fully-watered no alteration was due to the chemical treatment but they showed a significant decrease in their protein abundance because of the AM inoculation in plants submitted to SA application.

The relative abundance of ZmTIP1;1 aquaporin was unaltered by the studied parameters under well-watered conditions (Fig. 3F). Nevertheless, under water limitation, AM inoculation led to a significant increases in ZmTIP1;1 abundance when plants were either untreated or treated with AIP, and this effect was not found in SA-treated plants (Fig. 3F).

Fully watered plants did not show significant changes in ZmPIP2;4 relative abundance due to SA or AIP application and AM inoculation. These plants only featured enhanced aquaporin content when plants were AM inoculated and treated with AIP (Fig. 3G). Under drought conditions ZmPIP2;4 abundance was not significantly altered by chemical application, SA or AIP. In such circumstances AM inoculation led to significant increase of ZmPIP2;4 protein abundance in untreated plants.

3.5. Sap and root phytohormone contents

Sap IAA content under well-watered conditions was unaffected by any of the studied chemical treatment or even AM inoculation (Fig. 4A). However, under drought conditions a greater IAA content in non-AM plants was shown after SA application. In contrast, this chemical treatment showed a significant drop in sap IAA when plants were AM-inoculated. Sap ABA concentration featured no differences due to chemical treatment or fungal inoculation regardless of the water regime (Fig. 4B). Similar trend was registered for sap SA content in well-watered plants which were unaffected by chemical treatment (Fig. 4C). Nevertheless, under drought stress conditions, SA application provoked the sap SA content to increase in non AM plants. Remarkably, in such stressed plants a significant decrease in sap SA content was found due to AM inoculation. Sap JA concentration was unaffected by the studied hormone treatment when plants grew under full water regime (Fig. 4D) but under drought stress conditions, AM inoculation significantly increased JA levels of untreated plants.

Root IAA content was unaffected by chemical treatment or AM inoculation under well-watered conditions (Fig. 4E). Only in SA-treated plants AM inoculation significantly increased IAA concentration. However, when plants were submitted to water deficit, AM inoculation led to a significant root IAA increase in all chemical treatments, particularly after SA application.

Under well-watered conditions, plants featured no changes in root ABA concentration due to chemical application but a significant increase because of AM inoculation was observed in the case of untreated plants or plants treated with AIP (Fig. 4F). In droughted plants root ABA was not susceptible to change after chemical treatment but in all cases AM inoculation led to significant increases in ABA content.

Regarding the root SA concentration, plants were not significantly altered by chemical treatment regardless of the water regime (Fig. 4G), but AM-inoculated plants significantly increased their contents, except in the case of well-watered plants treated with SA. It was also highlighted that under well-watered conditions the combination of AM inoculation and AIP augmented root SA concentration.

Under fully-watered conditions AIP application significantly increased root JA content in non-AM plants (Fig. 4H). In such water regime *R. irregularis* led to root JA drop. Besides, when plants were submitted to drought stress, AIP application also promoted root JA accumulation in non-AM plants. However, under drought stress, no significant changes as consequence of AM inoculation were featured regarding root JA.

Root JA-Ile of well-watered plants presented increased content after AIP treatment in both AM-inoculated and non-inoculated plants (Fig. 4I), but when plants were submitted to drought treatment no significant changes were observed due to the chemical or fungal treatments.

3.6. Correlations among root hydraulic properties and the studied parameters

Under well-watered conditions hydrostatic root hydraulic conductivity (Lpr) was found to be negatively correlated with *ZmPIP2;4* and *ZmTIP1;1* gene expression as reflected by the Pearson correlation test (Table 2). However no correlation was found between osmotic root hydraulic conductivity (Lo) and the measured parameters. Also, none of the measured parameters showed significant correlation with the percentage of water flowing thought the

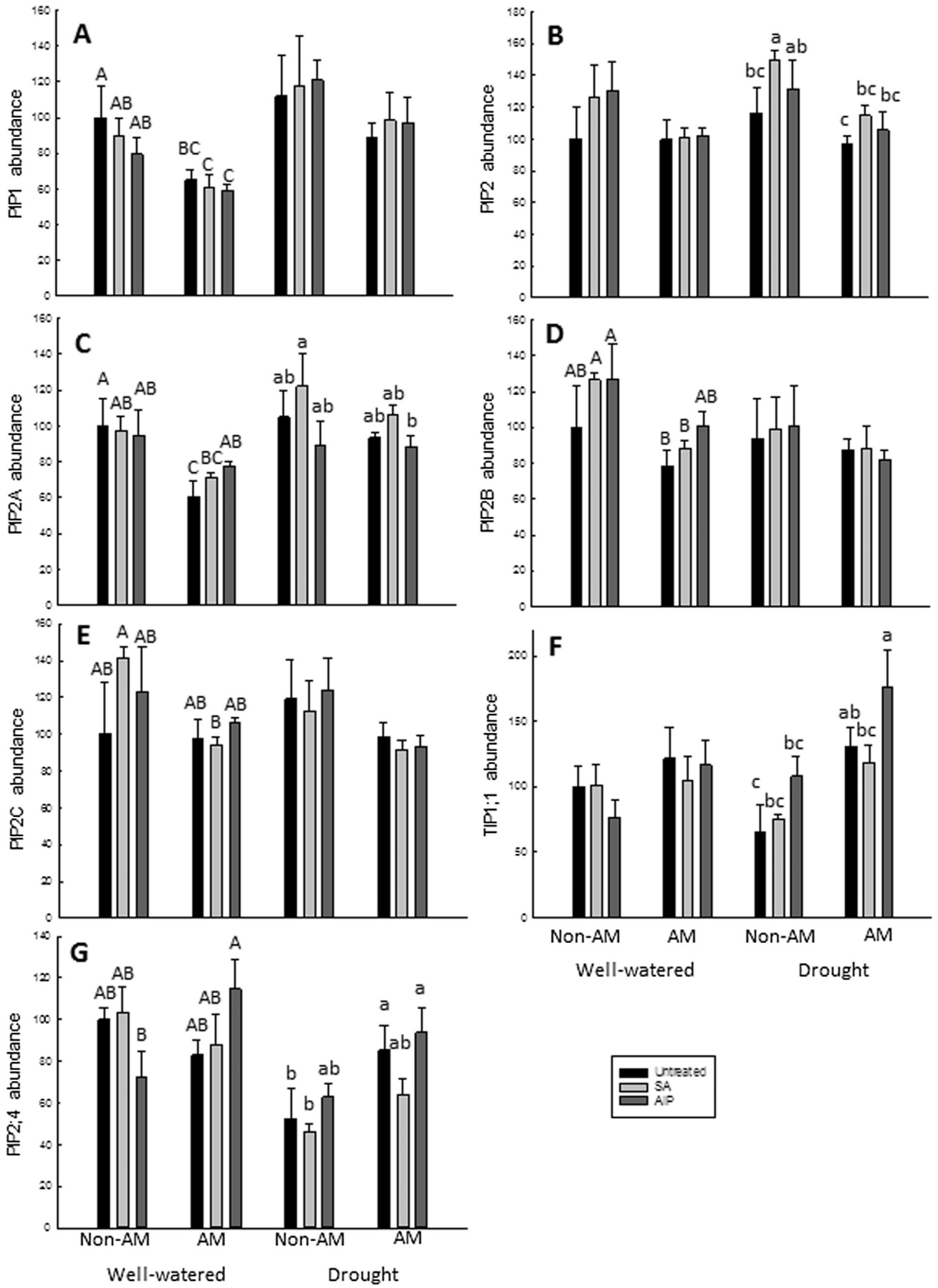

Fig. 3. PIP1 (A), PIP2 (B), PIP2A (C), PIP2 B (D), PIP2C (E), ZmTIP1;1 (F) and ZmPIP2;4 (G) relative protein abundance in maize plants inoculated (AM) or not (Non-AM) with the AM fungus *Rhizophagus irregularis* and submitted to two water regimes (well-watered or drought stress). Plants remained untreated or received exogenous salicylic acid (SA) or an inhibitor of SA biosynthesis (AIP). Data represents the means of five values ± SE. Different letter indicates significant differences among treatments ($p < 0.05$) based on Duncan's test for well-watered (uppercase) and drought stressed (lowercase) plants. The absence of letters indicates that no significant differences among treatments were found.

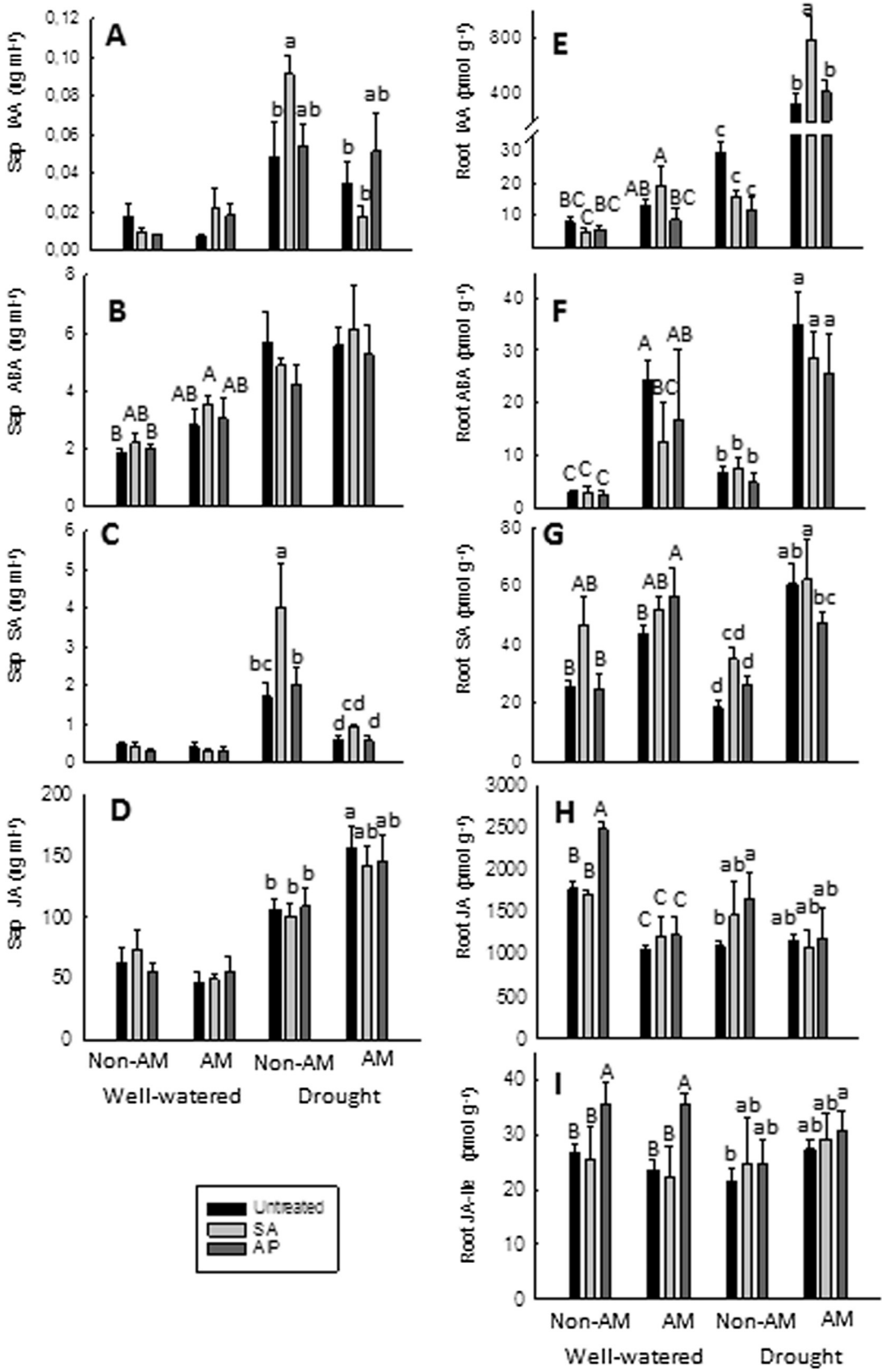

Fig. 4. IAA, ABA, SA and JA concentration in sap (A to D), and IAA, ABA, SA, JA and JA-Ile concentration in roots (E to I) in maize plants inoculated (AM) or not (Non-AM) with the AM fungus *Rhizophagus irregularis* and submitted to two water regimes (well-watered or drought stress). Plants remained untreated or received exogenous salicylic acid (SA) or an inhibitor of SA biosynthesis (AIP). Data represents the means of five values ± SE. Different letter indicates significant differences among treatments ($p < 0.05$) based on Duncan's test for well-watered (uppercase) and drought stressed (lowercase) plants. The absence of letters indicates that no significant differences among treatments were found.

Table 2
Pearson correlation coefficients between hydrostatic root hydraulic conductivity (Lpr), osmotic root hydraulic conductivity (Lo), relative apoplastic water flow, root and sap SA concentration and measured sap and root hormones, root aquaporin abundance and root aquaporin gene expression in well-watered and drought plants (n = 6). * Significant at $p < 0.05$; ** Significant at $p < 0.01$; *** Significant at $p < 0.00$.

		Well-watered				Drought			
		Lpr	Lo	% Apoplastic water flow	Sap SA	Lpr	Lo	% Apoplastic water flow	Sap SA
Sap hormones	ABA	0.577	−0.124	0.338	0.771	0.705	**0.836***	−0.235	**0.898***
	JA	0.739	−0.338	0.318	0.753	**0.876***	**0.962****	−0.188	0.798
	IAA	0.429	−0.231	0.123	**0.989****	0.792	0.733	0.263	0.235
	SA	0.472	−0.28	0.006	1	0.421	0.675	−0.612	1
Root hormones	ABA	0.489	−0.209	0.371	**0.862***	0.788	**0.851***	−0.309	0.666
	JA	−0.588	0.534	−0.75	−0.551	0.076	−0.145	0.728	−0.553
	JA-Ile	−0.629	0.64	−0.754	−0.487	0.213	0.322	−0.339	0.129
	IAA	0.316	0.052	0.585	0.464	0.496	0.73	−0.525	**0.987****
	SA	0.438	−0.773	−0.285	0.016	0.136	0.249	−0.202	0.542
Root protein abundance	PIP1	−0.607	−0.029	0.162	−0.776	**−0.876***	**−0.926*****	0.154	−0.739
	PIP2	−0.062	0.128	−0.664	−0.593	**−0.826***	**−0.894***	0.359	−0.572
	PIP2A	−0.521	0.033	−0.319	−0.734	−0.633	−0.646	0.219	−0.103
	PIP2B	−0.155	0.052	−0.663	−0.661	−0.808	**−0.901***	0.249	−0.694
	PIP2C	0.177	−0.226	−0.432	−0.632	−0.694	**−0.818***	0.195	**−0.857***
	PIP2;4	0.396	−0.606	0.249	0.019	**0.954****	**0.937****	0.005	0.404
	TIP1.1	0.6	−0.451	0.708	0.464	**0.836***	**0.872***	−0.041	0.474
Root gene expression	PiP1.1	−0.785	0.626	−0.51	−0.613	0.429	0.562	−0.564	0.499
	PIP1.3	−0.461	0.177	−0.6	−0.555	−0.545	−0.622	0.058	−0.532
	PIP2.2	−0.662	0.393	−0.559	−0.479	−0.076	−0.109	−0.195	−0.188
	PIP2.4	**−0.867***	0.391	−0.123	−0.742	−0.654	**−0.840***	0.394	**−0.855***
	TIP1.1	**−0.816***	0.633	−0.517	−0.513	−0.41	−0.417	−0.195	−0.315
	TIP2.3	−0.45	0.071	−0.346	−0.73	−0.681	−0.747	0.054	−0.629
	TIP4.1	−0.659	0.131	0.22	−0.685	−0.508	−0.648	0.643	−0.458
	NIP2.1	−0.469	−0.128	−0.002	−0.731	−0.61	−0.666	0.293	−0.248
Root hydraulic parameters	Lpr	1	−0.674	0.022	0.472	1	**0.938****	0.128	0.421
	Lo	−0.674	1	−0.288	−0.28	**0.938****	1	−0.199	0.675
	% Apoplastic water flow	0.022	−0.288	1	0.006	0.128	−0.199	1	−0.612

apoplastic route (Table 2). Our data also revealed the absence of correlation between root SA concentration and these variables. However, sap SA concentration was correlated positively with sap IAA and root ABA content (Table 2).

In contrast, under drought stress conditions Pearson correlation test revealed the positive correlation between Lpr and Lo (Table 2). Both measurements of root hydraulic conductivity, hydrostatic and osmotic, were significantly and positively correlated with sap JA concentration, ZmPIP2;4 and ZmTIP1;1 proteins abundance and negatively correlated with PIP1 and PIP2 proteins content. Besides, Lo also showed positive correlation with sap ABA concentration and root ABA content and was negatively correlated with PIP2 B and PIP2C proteins content, as well as, with *ZmPIP2;4* gene expression. Relative apoplastic water flow and root SA concentration did not correlate with any of the measured parameters. Contrariwise, sap SA concentration was positively correlated with sap ABA concentration and root IAA content, as well as, negatively correlated with PIP2C protein abundance and *ZmPIP2;4* gene expression (Table 2).

4. Discussion

In this study, we shed light on the differential root water transport regulation under water shortage when an AM fungus, in this case *R. irregularis*, is present in plant roots. Our group has already reported the modulation between different water transport pathways by the AM symbiosis in maize plants compared to non-inoculated plants under drought stress (Bárzana et al., 2012). Now we go further on this mechanism by studying for the first time the implication of external SA application in water transport regulation of AM plants.

4.1. AM enhances plant performance under drought

The water stress imposed in this study produced a drop of *gs* by around 75%, regardless of fungal AM inoculation (Table 1), as in isohydric cultivars this is one of the earliest responses to water deprivation (Hasanuzzaman et al., 2014). Indeed, the reduction in plant biomass production caused by drought stress has been related to direct effects on the plant photosynthetic capacity due to reduced stomatal conductance (Sheng et al., 2008). Moreover, drought stress caused a significant reduction in plant biomass production in all treatments, although AM plants always maintained higher values of SDW and RDW than non-AM plants. Thus, AM-improved drought tolerance in maize plants was firstly demonstrated by the higher biomass production by these plants under water deprivation treatment (Table 1). The positive effect of mycorrhization was also observed under well-watered conditions both in shoot and root dry weights (Table 1). The enhancement of drought tolerance in maize and other plant species by AM colonization involves greater plant biomass (Boomsma and Vyn, 2008) thanks in part to a better plant mineral nutrition (Smith and Smith, 2011). However, a higher capacity for CO_2 fixation in AM plants may also have accounted for this improved plant growth since enhanced Rubisco activity in AM grapevine and rice plants has been described under drought and salinity, respectively (Porcel et al., 2015; Valentine et al., 2006).

In this study, AM symbiosis did not lead to the enhancement of *gs* values probably due to the higher biomass of AM plants, which implies an also higher total transpiration rate in AM plants (Baslam et al., 2012). However, better membrane stability of droughted AM plants compared to non-AM ones was observed by measuring the percentage of electrolyte leakage (EL). In these plants, EL maintained the steady-state values of well-watered plants (Table 1). This effect of AM association is consistent with our previous observation under drought stress conditions (Quiroga et al., 2017).

4.2. Root water transport is regulated by AM and SA application

To cope with water scarcity, plants have developed a variety of strategies, including regulation of tissues permeability to water movement (Calvo-Polanco et al., 2016). Root hydraulic conductiv-

ity (Lpr) was measured as an estimation of the root water transport potential and to determine its role under limited water availability. Drought stress produced a drop of Lpr and Lo (Fig. 1A and B), often addressed in the literature under water deprivation or other abiotic stresses like salinity (Boursiac et al., 2005; Martínez-Ballesta et al., 2003; Martre et al., 2001; Meng and Fricke, 2017). Some authors argued that this phenomenon and the consequent decrease of water uptake by roots could be a mechanism for the avoidance of water loss when soils start to dry (Aroca et al., 2012). However, AM plants enhanced Lpr of droughted plants (Fig. 1A), and this positive effect was already observed in other studies (Sánchez-Romera et al., 2016), probably because these plants do not suffer dehydration as much as non-inoculated plants. Moreover, in this study the effects of the applied chemical compound are mainly observed in water stressed plants (Fig. 1A). This could be due to a different dynamic of droughted roots for water uptake as compared to well-watered roots, and therefore, to different efficiency for chemical uptake from the nutrient solution. SA decreased Lpr of droughted-AM plants, while the application of AIP maintained steady-state Lpr levels (Fig. 1A).

In addition, osmotic component of root hydraulic conductivity (Lo), that gives information of water flowing through the cell-to-cell pathway, where aquaporins participate (Maurel et al., 2008), presented the same trend under drought stress than Lpr (Fig. 1B), diminishing its levels when applying SA in both non-AM or AM plants. This suggests that SA may be also altering aquaporin regulation, as it was previously pointed by Boursiac et al. (2008) and Du et al. (2013). However, in this study the effects of SA on root hydraulic properties and aquaporin gene expression were not evident. The lack of a clear correlation between SA-mediated root hydraulic properties and SA-mediated aquaporin gene expression may be due to the fact that some aquaporins genes cannot be regulated by SA because of the lack of SA-responsive elements in their promoter region, as evidenced by Tungngoen et al. (2011) for a *Hevea brasilensis* PIP aquaporin. No information is available currently about the presence or absence of such elements in the promoter regions of the maize aquaporins. Moreover, a delay between hormonal treatment (IAA) and aquaporin gene expression has been described in Arabidopsis (Péret et al., 2012), which may also occur here with SA. Thus, the way through which SA regulates these membrane proteins is uncertain, and the two studies mentioned above presented contradictory results about the regulation of aquaporin internalization by the hormone. Du et al. (2013) found that increased SA levels hinder the constitutive recycling of membrane proteins like aquaporins, increasing the abundance of some of them in the plasmalemma, as a mechanism to control their activity. In contrast, Boursiac et al. (2008) described an stimulus-induced internalization of PIP proteins after SA application meditated by reactive oxygen species (ROS). In any case, aquaporin modulation was extensively reported to substantially contribute to total root water flow (Boursiac et al., 2008; Knipfer and Fricke, 2011; Martínez-Ballesta et al., 2000; Martre et al., 2001; Vandeleur et al., 2014). In our study, significant correlation between aquaporin accumulation and Lo was found exclusively under drought stress treatment (Table 2), but not under well-watered conditions. This supports the idea of ROS involvement, as they may accumulate under drought conditions, leading to relocalization of aquaporins as reported by independent studies (Boursiac et al., 2008; Velikanov et al., 2015).

Since plants undergo frequent environmental changes, the activity of aquaporins must be regulated by mechanisms that allow rapid responses to these changes. Aquaporins regulate cell water flow either through changes in their abundance or channel gating (Tyerman et al., 2002). Post-translational modifications are also necessary to achieve such rapid regulation (Vandeleur et al., 2014), including phosphorylation/de-phosphorylation of specific serine residues, the first post-translational regulation mechanism found in aquaporins (Prado et al., 2013; Prak et al., 2008). This modification generates conformational changes allowing aquaporin gating or modifying its subcellular localization in the membrane (Johansson et al., 1998; Prado et al., 2013; Prak et al., 2008) and has been proposed as a mechanism to prevent water loss (Bárzana et al., 2015). Phosphorylation of C-terminal residues Ser-280 and Ser-283 of PIP2 aquaporins was correlated to the regulation of Lpr in plants (Prado et al., 2013). The present data show that PIP2 B (Ser-283) and PIP2C (Ser-280 and Ser-283) both negatively correlated with Lo under DS, as well as PIP2 and PIP1 protein levels (Table 2). However, when analysing ZmPIP2;4 root aquaporin abundance, one of the most abundant aquaporins in maize roots, with prominent role in water transport (Chaumont et al., 2001), it correlated positively with Lo under water shortage (Table 2). This could be contradictory, but it must be taken into account that the PIP2 antibody recognizes several different isoforms within the PIP2 subfamily, that may have different roles in water transport regulation. ZmTIP1;1, protein abundance presented the same trend previously described for ZmPIP2;4 protein and consequently also correlated with Lpr (Table 2), suggesting that these two proteins could be of high interest in regulating water transport in our experimental conditions. Surprisingly, *ZmPIP2;4* transcript levels negatively correlated with Lo (Table 2). However, expression levels of aquaporins do not always correlate with their protein abundance, as both change along time and with the growing conditions (Chaumont and Tyerman, 2014). Expression patterns of most analysed aquaporins differed in AM and non-AM plants (Fig. 2), which in general involved their downregulation in AM plants, as was shown in previous reports (Bárzana et al., 2014; Quiroga et al., 2017).

Interestingly, it has been highlighted the different behaviour of non-AM and AM plants under drought after SA application. Whereas non-AM plants increased the percentage of apoplastic water flow in presence of exogenous SA, plants inoculated with the AM fungus decreased water circulating through this pathway when the hormone was applied (Fig. 1C). This effect can result from the differential effect of this hormone on Lpr in plants inoculated with the symbiotic fungus under drought. This is consistent with previous results, where AM plants were suggested to have a higher plasticity for switching between water transport pathways (Bárzana et al., 2012). In addition, this differential effect of SA on the apoplastic water flow in AM and non-AM plants may be mediated by altered nitric oxide (NO) content in these plants, since it has been recently shown that SA-induced NO regulates maize water content and hydraulic conductivity under drought (Shan and Wang, 2017). Moreover, Sánchez-Romera et al. (2017) have suggested that NO favours apoplastic water pathway inside roots and suggested that different outcomes in root hydraulic conductivity observed between AM and non-AM plants could be mediated by differences in NO content. Thus, a higher NO content in non-AM plants than in AM ones could explain the SA-induced enhancement of apoplastic water flow in non-AM plants and the opposite effect in AM plants.

4.3. Implication of phytohormones in root water transport regulation

Salicylic acid has been previously shown to alter plant water relations under drought or salt stress conditions (Faried et al., 2017; Farooq et al., 2010; Khan et al., 2015). On the light of our results, no clear relationship between exogenous SA application and root or sap SA concentration increase was found, although in sap, an enhancement in such hormone occurred under drought (Fig. 4C, G and L). However, it is noteworthy that, as mentioned above, SA effects on plant functions are dose-dependent (Khan et al., 2015; Miura and Tada, 2014). Indeed, an important aspect regarding the effect of SA application is the dose of SA and the

method of application (via foliar or via hydroponic solution). Generally, low concentrations (less than 0.5 mM) of SA increase drought tolerance, while high concentrations (2–3 mM) decrease drought tolerance (Miura and Tada, 2014). Thereby, the selected dose in this study (0.02 mM, applied via hydroponic solution) is enough to affect Lpr, but may not be sufficient to alter hormonal tissue content. Nonetheless, SA could be modifying Lpr through the alteration of other phytohormones in roots, as a complex crosstalk among these compounds may take place, controlling plant performance under different environmental conditions (Munné-Bosch and Müller, 2013). In fact, a consistent response in AM plants was the increase of IAA, ABA and SA in roots under drought conditions compared to non-AM plants (Fig. 4E, F and G). In this sense, SA has been described to play a role in the regulation of AM root colonization, although the precise mechanism is not clear yet (Herrera-Medina et al., 2003). Moreover, SA was also reported to induce genes involved in ABA biosynthesis, as well as to modify ABA transport to the shoots (Horváth et al., 2015). Regarding the functioning of the AM symbiosis, ABA was related to arbuscule formation, thus being necessary for efficient AM symbiosis establishment and functioning (Miransari et al., 2012). An enhancement of the ABA content by the AM symbiosis was clearly reflected by our results (Fig. 4F). Taking together these data and the previously described enhancement of Lpr by ABA (Aroca et al., 2008; Parent et al., 2009) we hypothesize that enhanced ABA in AM inoculated plants may favour the increase in root conductivity under drought conditions. This idea is also supported by the higher Lpr and Lo found in AM plants under drought stress (Fig. 1A and B). In addition to this, IAA is considered to be essential for AM infection, especially during pre-symbiotic interactions (Hanlon and Coenen, 2011) and our result showed an important enhancement of root IAA content after AMF root colonization, especially in SA-treated plants. This enhanced IAA levels in AM SA-treated plants may have contributed to the reduction in the hydraulic parameters measured here, since Péret et al. (2012) showed that exogenous IAA application inhibited most aquaporins genes in *A. thaliana* and reduced root hydraulic conductivity both at the cell and whole-organ level. In relation with AM development, some authors also found alterations in root JA levels with root colonization (Liu et al., 2013; Pedranzani et al., 2016). However, no changes were found in other studies (López-Ráez et al., 2010; Sánchez-Romera et al., 2016).

ABA, JA and SA were hypothesized to have some common regulatory elements in their signalling pathways, although their clear relationship was not established yet (Proietti et al., 2013). SA and JA interaction has normally been reported to be antagonistic in defence response (Koornneef et al., 2008). Although from hormonal content we cannot observe any clear relationship between them, sap JA levels positively correlated with Lo and Lpr under drought (Table 2) and this is in line with previous results of JA on Lpr (Sánchez-Romera et al., 2014, 2016). An opposite effect was induced by SA on these parameters, which agree with results by Volobueva et al. (2004), who reported decreased water conductance in maize roots by SA addition and results by Boursiac et al. (2008) showing down-regulation of root water transport by SA in Arabidopsis plants. Thus, from our data we could deduce that there is a relationship between these hormones in response to drought, which is regulated by AM colonization, even if further research is needed to explain accurately the way these hormones interact.

5. Conclusions

In the present work we demonstrated that AM symbiosis can modify root hydraulic response to drought episodes. Under these conditions, AM plants showed both increased Lpr and Lo. This, together with the better exploration and exploitation of the soil water resources by the fungal hyphae that has been widely described in literature (Marulanda et al., 2003; Allen, 2007, 2009; Ruth et al., 2011), may results in greater amount of water available to the AM plants and better performance of AM plants under water deprivation.

Exogenous SA application altered root hydraulic parameters, decreasing Lpr and Lo under drought, while application of its inhibitor, AIP maintained steady state levels for these parameters. SA modulation of water conductivity could be due to a fine-regulation of root aquaporins (as ZmPIP2;4 or ZmTIP1;1). Furthermore, SA application differently modulated the percentage of water flowing by the apoplastic pathway under the imposed stress, decreasing its contribution to total root water flow in AM plants and increasing it in non-AM plants.

Intricate relationship between Lpr, aquaporins and endogenous levels of phytohormones, especially SA, ABA and JA was observed, revealing a complex network controlling water transport in roots. Future researches should analyse the promoter regions of the maize aquaporin genes to search for hormone responsive elements and to explore more in detail the crosstalk mechanism between these hormones in the regulation of water transport in AM roots, in order to better understand the mechanism through which the AM symbiosis copes with root dehydration and contributes to improved root hydraulic properties under drought conditions.

Acknowledgements

This work was financed by Ministerio de Economía y Competitividad (Project AGL2014-53126-R). GQ is a FPI Ph.D. Grant Holder. We thank DuPont Pioneer Corporation for providing us the maize seeds.

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